PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 August 1999 (19.08.99)	
International application No. PCT/US98/25720	**Applicant's or agent's file reference** F126422
International filing date (day/month/year) 11 December 1998 (11.12.98)	**Priority date** (day/month/year) 12 December 1997 (12.12.97)
Applicant NALDINI, Luigi et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   24 June 1999 (24.06.99)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marie McAdams
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

TENT COOPERATION TR

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DEAN H. NAKAMURA
SUGHRUE, MION, ZINN, MACPEAK &
SEAS, PLLC
2100 PENNSYLVANIA AVE., N.W., SUITE 800
WASHINGTON DC 20037-3213

PCT

NOTIFICATION OF RECEIPT
OF DEMAND

(PCT Rule 61.1(b), first sentence
and Administrative Instructions, Section 601)

Date of mailing *(day/month/year)*	21 JUL 1999
Applicant's or agent's file reference: F126422	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/25720	11 DEC 98	12 DEC 97

Applicant: NALDINI, LUIGI

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

24 June 1999 (24.06.99)

2. This date of receipt is:

☑ the actual date of receipt of the demand.

☐ the date on which the proper corrections to the demand were timely received.

3. ☐ This date is **AFTER** the expiration of 19 months from the priority date.

Attention: The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.

☐ This notification confirms the information given in person or by telephone on:

_____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/US	Authorized officer
Assistant Commissioner for Patents Box PCT Washington, D.C. 20231 Attn: IPEA/US Facsimile No.	Jeannette Washington PCT Operations - IAPD Team 1 Telephone No. (703) 305-3687 (703) 305-3230 (FAX)

Form PCT/IPEA/402 (July 1992)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: DEAN H. NAKAMURA
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 PENNSYLVANIA AVENUE, N.W., SUITE 800
WASHINGTON DC 20037-3213

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference F126422	Date of Mailing *(day/month/year)* 25 FEB 1999 **FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US98/25720	International filing date *(day/month/year)* 11 DECEMBER 1998
Applicant NALDINI, LUIGI	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ANNE-MARIE BAKER, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/220 (January 1994)★

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference F126422	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US98/25720	International filing date *(day/month/year)* 11 DECEMBER 1998	(Earliest) Priority Date *(day/month/year)* 12 DECEMBER 1997
Applicant NALDINI, LUIGI		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [ ] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25720

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 48/00, 35/00; C12N 15/63
US CL :514/44; 424/93.1; 435/320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/93.1; 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,650,309 A (WONG-STAAL et al.) 22 July 1997, see entire document, especially column 2 and column 15, lines 54-60.	1-4
X	LISZIEWICZ et al. Inhibition of human immunodeficiency virus type 1 replication by regulated expression of a polymeric Tat activation response RNA decoy as a strategy for gene therapy in AIDS. PNAS USA, September 1993, Vol. 90, pages 8000-8004, see entire document.	1-4
A	BERKHOUT et al. Tat trans-activates the human immunodeficiency virus through a nascent RNA target. Cell 20 October 1989, Vol. 59, pages 273-282, entire document.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
12 FEBRUARY 1999

Date of mailing of the international search report
25 FEB 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
ANNE-MARIE BAKER, PH.D.
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25720

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS
DIALOG (file:medicine, USPatFull, Derwent, European Patents, JAPIO)
search terms: HIV, lentivir?, retrovir?, vector?, LTR, treat?, infect?

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 48/00, 35/00, C12N 15/63

A1

(11) International Publication Number: **WO 99/30742**

(43) International Publication Date: 24 June 1999 (24.06.99)

(21) International Application Number: PCT/US98/25720

(22) International Filing Date: 11 December 1998 (11.12.98)

(30) Priority Data:
60/069,579 12 December 1997 (12.12.97) US

(71)(72) Applicants and Inventors: NALDINI, Luigi [IT/IT]; Corso Monte Cucco 144, I-10141 Torino (IT).. SONG, Jin-Ping [CN/US]; 3455 Rambow Drive, Palo Alto, CA 84306 (US).

(74) Agents: NAKAMURA, Dean, H. et al.; Sughrue, Mion, Zinn, Macpeak & Seas, PLLC, Suite 800, 2100 Pennsylvania Avenue, N.W., Washington, DC 20037-3213 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: THERAPEUTIC USE OF LENTIVIRAL VECTORS

(57) Abstract

A lentivirus vector inhibits propagation of a lentivirus in a cell.

THERAPEUTIC USE OF LENTIVIRAL VECTORS

FIELD OF THE INVENTION

The invention relates to the use of lentiviral vectors in the treatment of a disease resulting from or associated with a lentivirus.

BACKGROUND OF THE INVENTION

Retrovirus vectors are a common tool for gene delivery (Miller, Nature (1992) 357:455-460). The ability of retrovirus vectors to deliver an unrearranged, single copy gene into a broad range of rodent, primate and human somatic cells makes retroviral vectors well suited for transferring genes to a cell.

Lentiviruses are complex retroviruses which, in addition to the common retroviral genes gag, pol and env, contain other genes with regulatory or structural function. The higher complexity enables the lentivirus to modulate the life cycle thereof, as in the course of latent infection.

A typical lentivirus is the human immunodeficiency virus (HIV), the etiologic agent of AIDS. In vivo, HIV can infect macrophages, which are terminally differentiated cells that rarely divide. In vitro, HIV can infect primary cultures of monocyte-derived macrophages (MDM) as well as HeLa-Cd4 or T lymphoid cells arrested in the cell cycle by treatment with aphidicolin or γ irradiation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

SUMMARY OF THE INVENTION

The instant invention relates to the use of lentiviral vectors per se for a therapeutic benefit. The vector need not contain a transgene with antiviral activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts four graphs of Gag p24 antigen expression in human SupT1 lymphocytes transduced with lentiviral vector at different multiplicity of infection (M.O.I.; rectangles, triangles, ellipses) or in control non-transduced cells (lozenges) after infection with different amounts of HIV.

Figure 2 depicts Gag p24 antigen expression and cell survival after HIV infection of human primary $CD4^+$ lymphocytes transduced with either a lentiviral vector (triangles) or a murine leukemia virus based vector (squares) or non-transduced cells (diamonds).

DETAILED DESCRIPTION OF THE INVENTION

The instant invention provides use of a lentiviral vector. The vector can be one which carries a foreign gene with an anti-viral activity, however, that is not a prerequisite in the practice of the instant invention. Thus, a vector per se can be used.

The lentiviral genome and the proviral DNA have the three genes found in retroviruses: gag, pol and env, which are flanked by two long terminal repeat (LTR) sequences. The gag gene encodes the internal structural (matrix, capsid and nucleocapsid) proteins; the pol gene encodes the RNA-directed DNA polymerase (reverse transcriptase), a protease and an integrase; and the env gene encodes viral envelope glycoproteins. The 5′ and 3′

LTR's serve to promote transcription and polyadenylation of the virion RNA's. The LTR contains all other cis-acting sequences necessary for viral replication. Lentiviruses have additional genes including vif, vpr, tat, rev, vpu, nef and vpx (in HIV-1, HIV-2 and/or SIV).

Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient encapsidation of viral RNA into particles (the Psi site). If the sequences necessary for encapsidation (or packaging of retroviral RNA into infectious virions) are missing from the viral genome, the cis defect prevents encapsidation of genomic RNA. However, the resulting mutant remains capable of directing the synthesis of all virion proteins.

The vectors of interest are those which have an intact 5' and 3' lentivirus LTR. A vector of interest contains a packaging signal sequence comprising the leader sequence downstream of the LTR and until the beginning of the gag gene. The vector may also contain an additional portion of the gag gene to enhance packaging. The vector of interest also includes a part of the env gene containing the Rev Response Element (RRE), and it may or may not include a splice acceptor site downstream of the RRE. The vectors of interest may contain one or more transgenes, or foreign nucleic acid, and preferably a transgene with anti-viral activity. However, a vector of interest need not contain a heterologous gene.

The heterologous or foreign nucleic acid sequence, the transgene, is linked operably to a regulatory nucleic acid sequence. As used herein, the term "heterologous" nucleic acid sequence refers to a sequence that originates from a foreign species, or, if from the same species, it may be substantially modified from the original form. Alternatively, an unchanged nucleic acid sequence that is

not expressed normally in a cell is a heterologous nucleic acid sequence.

The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. Preferably, the heterologous sequence is linked to a promoter, resulting in a chimeric gene. The heterologous nucleic acid sequence is preferably under control of either the viral LTR promoter-enhancer signals or of an internal promoter, and retained signals within the retroviral LTR can still bring about efficient expression of the transgene.

The foreign gene can be any transcribable nucleic acid of interest. Generally the foreign gene encodes a polypeptide. Preferably the polypeptide has some therapeutic benefit. The polypeptide may supplement deficient or nonexistent expression of an endogenous protein in a host cell. The polypeptide can confer new properties on the host cell, such as a chimeric signalling receptor, see U.S. Pat. No. 5,359,046. The artisan can determine the appropriateness of a foreign gene practicing techniques taught herein and known in the art. For example, the artisan would know whether a foreign gene is of a suitable size for encapsidation and whether the foreign gene product is expressed properly.

It may be desirable to modulate the expression of a gene regulating molecule in a cell by the introduction of a molecule by the method of the invention. The term "modulate" envisions the suppression of expression of a gene when it is over-expressed or augmentation of expression when it is under-expressed. Where a cell proliferative disorder is associated with the expression of a gene, nucleic acid sequences that interfere with the expression of a gene at the translational level can be

used. The approach can utilize, for example, antisense nucleic acid, ribozymes or triplex agents to block transcription or translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving same with a ribozyme. The target of those molecules is the lentiviral RNA. Moreover, the RNA may be a sequence of the virus not present in the vector or that has been mutated in the vector.

Antisense nucleic acids are DNA or RNA molecules which are complementary to at least a portion of a specific mRNA molecule (Weintraub, Sci. Am. (1990) 262:40). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides or more are preferred since such are synthesized easily and are less likely to cause problems than larger molecules when introduced into the target cell. The use of antisense methods to inhibit the in vitro translation of genes is well known in the art (Marcus-Sakura, Anal. Biochem. (1988) 172:289).

The antisense nucleic acid can be used to block expression of a viral protein or a dominantly active gene product, such as amyloid precursor protein that accumulates in Alzheimer's disease. Such methods are also useful for the treatment of Huntington's disease, hereditary Parkinsonism and other diseases. Antisense nucleic acids are also useful for the inhibition of expression of proteins associated with toxicity.

Use of an oligonucleotide to stall transcription can be by the mechanism known as the triplex strategy since the oligomer winds around double-helical DNA, forming a

three-strand helix. Therefore, the triplex compounds can be designed to recognize a unique site on a chosen gene (Maher et al., Antisense Res and Dev. (1991) 1(3):227; Helene, Anticancer Drug Dis. (1991) 6(6):569).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode those RNA's, it is possible to engineer molecules that recognize and cleave specific nucleotide sequences in an RNA molecule (Cech, J. Amer. Med Assn. (1988) 260:3030). A major advantage of that approach is only mRNA's with particular sequences are inactivated.

It may be desirable to transfer a nucleic acid encoding a biological response modifier. Included in that category are immunopotentiating agents including nucleic acids encoding a number of the cytokines classified as "interleukins", for example, interleukins 1 through 12. Also included in that category, although not necessarily working according to the same mechanism, are interferons, and in particular gamma interferon (γ-IFN), tumor necrosis factor (TNF) and granulocyte-macrophage colony stimulating factor (GM-CSF). It may be desirable to deliver such nucleic acids to bone marrow cells or macrophages to treat inborn enzymatic deficiencies or immune defects. Nucleic acids encoding growth factors, toxic peptides, ligands, receptors or other physiologically important proteins also can be introduced into cells. The transgene also can be an inducible toxic molecule.

The method of the invention may also be useful for neuronal, glial, fibroblast or mesenchymal cell transplantation, or "grafting", which involves transplantation of cells infected with the recombinant lentivirus of the invention ex vivo, or infection in vivo

into the central nervous system or into the ventricular cavities or subdurally onto the surface of a host brain. Such methods for grafting will be known to those skilled in the art and are described in Neural Grafting in the Mammalian CNS, Bjorklund & Stenevi, eds. (1985).

For diseases due to deficiency of a protein product, gene transfer could introduce a normal gene into the affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For example, it may be desirable to insert a Factor VIII or IX encoding nucleic acid into a lentivirus for infection of a muscle, spleen or liver cell.

The promoter sequence may be homologous or heterologous to the desired gene sequence. A wide range of promoters may be utilized, including a viral or a mammalian promoter, and an inducible promoter. Cell or tissue specific promoters can be utilized to target expression of gene sequences in specific cell populations. Suitable mammalian and viral promoters for the instant invention are available in the art.

Optionally during the cloning stage, the nucleic acid construct referred to as the transfer vector, having the packaging signal and the heterologous cloning site, also contains a selectable marker gene. Marker genes are utilized to assay for the presence of the vector, and thus, to confirm infection and integration. The presence of a marker gene ensures the selection and growth of only those host cells which express the inserts. Typical selection genes encode proteins that confer resistance to antibiotics and other toxic substances, e.g., histidinol, puromycin, hygromycin, neomycin, methotrexate etc. and cell surface markers.

The recombinant virus of the invention is capable of

transferring a nucleic acid sequence into a mammalian cell. The term, "nucleic acid sequence", refers to any nucleic acid molecule, preferably DNA, as discussed in detail herein. The nucleic acid molecule may be derived from a variety of sources, including DNA, cDNA, synthetic DNA, RNA or combinations thereof. Such nucleic acid sequences may comprise genomic DNA which may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with promoter regions, poly A sequences or other associated sequences. Genomic DNA may be extracted and purified from suitable cells by means well known in the art. Alternatively, messenger RNA (mRNA) can be isolated from cells and used to produce cDNA by reverse transcription or other means.

Preferably, the recombinant lentivirus produced by the method of the invention is a derivative of human immunodeficiency virus (HIV).

The vectors of interests are produced using known methods. The vectors of interest can be introduced into cells either as the nucleic acid or encapsidated as a virus particle. An artisan is familiar with methods for encapsidating a lentiviral vector of interest. The vectors are introduced into target cells using methods known by those of skill in the art.

Thus, the vectors can be introduced into human cell lines by calcium phosphate transfection, lipofection or electroporation, generally together with a dominant selectable marker, such as neo, DHFR, Gln synthetase or ADA, followed by selection in the presence of the appropriate drug and isolation of clones. The selectable marker gene can be the transgene.

A likely means for transforming host cells with a vector of interest is by infecting cells with virus

particles carrying a vector of interest. Thus, the vector of interest would be encapsidated using known packaging systems, such as that taught in U.S. Pat. No. 5,686,279 and in Naldini et al. Science (1996) 272:263-267. Briefly, using either a stable packaging cell line or by transient transfection, the vector of interest is introduced into a cell which packages the vector of interest into viral particles. The virus particles are obtained from the culture medium, treated as known in the art to provide a virus preparation.

The target cell then is exposed to the virus preparation. That can be via in vivo administration means, wherein the virus preparation is administered to a host, for example, in a parenteral form. Alternatively, cells from the host can be retrieved and maintained in culture where those cells are exposed to the virus preparation. Once transformed, stably or not, the cells then can be returned to the host.

While the therapeutic benefit of the instant invention can be obtained by the vector per se, it is preferred that the vector carry a transgene. Preferably that transgene is one which itself has a therapeutic effect. Thus, the vectors of interest should have a place in current therapy of diseases associated with lentivirus.

Although the techniques used to construct vectors and the like are provided in standard resource materials which describe specific conditions and procedures, for convenience, the following paragraphs may serve as a guideline.

Construction of the vectors of the invention employs standard ligation and restriction techniques which are well understood in the art (see Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring

Harbor Laboratory, N.Y., 1982). Isolated plasmids, DNA sequences or synthesized oligonucleotides are cleaved, tailored and religated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are understood in the art, and the particulars of which are specified by the manufacturer of the commercially available restriction enzymes, see, e.g. New England Biolabs, Product Catalog. In general, about 1 μg of plasmid or DNA sequences is cleaved by one unit of enzyme in about 20 μl of buffer solution. Typically, an excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, which may be followed by ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in Methods of Enzymology 65:499-560 (1980).

Restriction cleaved fragments may be blunt ended by treating with the large fragment of E. coli DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTP's) using incubation times of about 15 to 25 minutes at 20°C in 50 mM Tris (pH 7.6) 50 mM NaCl, 6 mM $MgCl_2$, 6 mM DTT and 5-10 μM dNTP's. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTP's are present. If desired, selective repair can be performed by supplying only one of the dNTP's, or with selected dNTP's, within the limitations dictated by the nature of the sticky ends. After treatment with

Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or Bal-31 results in hydrolysis of any single-stranded portion.

Ligations can be performed in 15-50 μl volumes under the following standard conditions and temperatures: 20 mM Tris-Cl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 33 mg/ml BSA, 10 mM-50 mM NaCl and either 40 μM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0°C (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14°C (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 33-100 μg/ml total DNA concentrations (5-100 mM total end concentration). Intermolecular blunt end ligations (usually employing a 10-30 fold molar excess of linkers) are performed at 1 μM total ends concentration.

Lentiviral vectors (Naldini et al., supra and Proc. Natl. Acad. Sci. (1996) 93:11382-11388) have been used to infect human cells growth-arrested in vitro and to transduce neurons after direct injection into the brain of adult rats. The vector was efficient at transferring marker genes in vivo into the neurons and long term expression in the absence of detectable pathology was achieved. Animals analyzed ten months after a single injection of the vector, the longest time tested so far, showed no decrease in the average level of transgene expression and no sign of tissue pathology or immune reaction. (Blomer et al., J. Virol. (1997) 71:6641-6649). An improved version of the lentiviral vector in which the HIV virulence genes env, vif, vpr, vpu and nef were deleted without compromising the ability of the vector to transduce non-dividing cells have been developed. The multiply attenuated version represents a substantial improvement in the biosafety of the vector (Zufferey et al. Nat. Biotech. (1997) 15:871-875).

Viral supernatants are harvested using standard techniques such as filtration of supernatants 48 hours post transfection. The viral titer is determined by infection of, for example, 10^6 NIH 3T3 cells or 10^5 HeLa cells with an appropriate amount of viral supernatant, in the presence of 8 μg/ml polybrene (Sigma Chemical Co., St. Louis, MO). Forty-eight hours later, the transduction efficiency is assayed.

While not wanting to be bound to any posited hypothesis, it is believed the mechanism of the resistance was mapped to a post-integration step and shown to be dependent on an intact HIV LTR in the vector. On HIV infection of transduced cells, transcription from the vector LTR was enhanced greatly, resulting in increased expression of the transgene. Conceivably the vector RNA competes effectively with the viral RNA's both for binding the transactivators and for packaging by the budding viral particles, resulting in inhibition of viral replication and mobilization and spreading of the vector. Viral particles collected from the infected transduced cells were less infectious than virus collected from infected non-transduced cells, and transferred efficiently the transgene into naive cells.

Thus, expression of both the vector and the virus in the same cell is detrimental to viral replication, and result in mobilization and spreading of the transgene into selected target cells of HIV. That effect and the strong enhancement of transgene expression induced by HIV are significant advantages of an HIV-derived vector of anti-HIV gene therapy applications.

Thus, the instant vector will find use alone, either containing a transgene or not, and preferably the transgene has an antiviral activity; or in combination with another vector carrying a transgene with antiviral

activity, wherein the instant vector does or does not contain a transgene.

The viral particles can be further purified from the viral supernatants as known in the art.

The viral particles or vector nucleic acid can be administered to a host with a disorder associated with or caused by a lentivirus using known techniques.

Actual delivery of the vectors or particles is accomplished by using any physical method that will transport same into a host and into the target cell. As used herein, "vector", means both a bare recombinant lentiviral vector and recombinant lentiviral particle. Simply dissolving a vector in Hanks' balanced saline solution or phosphate buffered saline is sufficient to provide a solution useful for injection. There are no known restrictions on the carriers or other components that can be coadministered with the vector (although compositions that degrade the virion or polynucleotides thereof should be avoided in the normal manner with vectors).

Pharmaceutical compositions can be prepared as injectable formulations to be delivered intramuscularly, including implantable pumps (known by those of skill in the art and described, for example, in U.S. Pat. No. 5,474,552). Numerous formulations for injection are known and can be used in the practice of the instant invention. The vectors can be used with any pharmaceutically acceptable carrier for ease of administration and handling.

Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of the vector as a free acid (DNA

contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. A dispersion of viral particles also can be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, the preparations contain a preservative to prevent the growth of microorganisms. The sterile aqueous media employed are obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that administration by a syringe is possible. The formulation must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the vector in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

The therapeutic compounds of this invention may be administered to a host alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

The dosage of the instant therapeutic agents which will be most suitable for prophylaxis or treatment will vary with the form of administration, the particular recombinant vector chosen and the physiological characteristics of the particular patient under treatment. Generally, small dosages will be used initially and, if necessary, will be increased by small increments until the optimum effect under the circumstances is reached. Exemplary dosages are within the range of 10^8 up to approximately 5×10^{15} particles in a total volume of 3-10 ml.

The invention now having been described in detail, provided hereinbelow are non-limiting examples

demonstrating various embodiments of the instant invention.

Example 1

CONSTRUCTION OF THE LENTIVIRAL VECTORS

The lentiviral transfer vector plasmids were derived from the plasmid pHR'-CMV-LacZ described previously in Naldini et al., Science (1996) 272:263-267. Plasmid pHR'-CMV-Neo was derived by substituting the BamHI-XhoI fragment of pHR'-CMV-LacZ containing the E.coli LacZ gene with a BamHI-XhoI fragment containing the neomycin phosphotransferase gene of E.coli (Beck et al., Gene (1982) 19:327-336).

pHR2 is a lentiviral transfer vector in which 124 base pairs (bp) of nef sequences upstream of the 3' LTR in pHR have been replaced with a polylinker both to reduce HIV-1 sequences and to facilitate transgene cloning. pHR2 was derived from pHR'-CMV-LacZ by replacing the 4.6 kilobase (kb) ClaI-StuI fragment with an 828 bp ClaI-StuI fragment generated by PCR using pHR'-CMV-LacZ as the template and with the oligonucleotide primer, 5'-CCATCGATCACGAGACTAGTCCTACGTATCCCCGGGGACGGGATCCGCGGAATTCC GTTTAAGAC-3' (SEQ ID NO:_____) and the primer 5'-TTATAATGTCAAGGCCTCTC-3' (SEQ ID NO:_____) in a three part ligation with a 4.4 kb StuI-NcoI fragment and a 4.5 kb NcoI-ClaI fragment from pHR'-CMV-LacZ.

Plasmid pHR2-PGK-GFP was derived by cloning the XhoI-BamHI fragment of pRT43.3PGKF3 (WO 97/07225) containing the human PGK promoter (GenBank Accession number #M11958 nucleotides 2-516) and the BamHI-NotI fragment of plasmid of pEGFP1 (Clontech) containing a codon usage-optimized and improved version of the Green Fluorescent Protein (GFP) of A. victoria and a NotI-SacII linker, into the Xhol and SacII sites of pHR2.

Example 2

INHIBITION OF HIV-1 REPLICATION OF LYMPHOCYTES TRANSDUCED BY THE LENTIVIRAL VECTOR

Human SupT1 T-lymphoblastoid cells were obtained by ATCC. Human $CD4^+$ primary blood lymphocytes (PBL) were separated from buffy coats from donations, stimulated with 2.5 μg/ml phytohemagglutinin or Dynal beads coated with OKT3 and CD28 antibodies for 2 days, then washed and cultured with 100 U/ml of interleukin 2 (Chiron) in AIM-V medium (Gibco). The SupT1 cells or PBL were transduced either with lentivector or a murine leukemia virus (MLV) vector carrying the same transgene overnight in the presence of 2 μg/ml polybrene, then washed and selected for transgene expression after 48 hrs.

All vectors were produced by transient transection of 293T cells and pseudotyped with the VSV.G envelope as described previously (Naldini et al., Proc. Natl. Acad. Sci. (1996) 93:11382-88). Cells transduced with vectors carrying the neomycin resistance gene were selected in medium containing 1 mg/ml G418, then cultured in normal medium for virus challenge. Cells transduced with vectors carrying the green fluorescent protein (GFP) as transgene were selected by cell sorting.

The cells were challenged with increasing amounts of HIV virus. HIV-1 virions were produced either by 293T cells transfected with the proviral infectious molecular clone R8, or by SupT1 cells chronically infected with R8 virus. R8 is a lymphocytotropic HIV-1 hybrid of the HXB2-D and NL43 isolates that expresses all HIV reading frames (Gallay et al., Cell (1995) 83:569-576). The virus stock was titered on HeLa P4 cells and had an infectivity of 1,000 to 3,000 infectious units/ng p24. The cells were washed twice after overnight incubation with the virus in the presence of 2 μg/ml polybrene, and further cultured

for up to 3 weeks. Every 3-4 days, the conditioned medium was collected and HIV replication was determined by accumulation of HIV-1 Gag p24 in the medium by a commercially available ELISA kit (DuPont).

In the first experiment (see Figure 1), SupT1 cells transduced by lentiviral vector carrying the neomycin resistance gene, pHR'-CMV-Neo, were tested. HIV accumulated in control non-transduced cultures. On the other hand, in cells transduced by the lentiviral vector, pHR2, HIV replication was detected only for the higher amounts of HIV and p24 accumulation was decreased dramatically and delayed. Similar results were obtained with three different SupT1 populations selected after transduction with the lentiviral vector at different multiplicity of infection (M.O.I.). Moreover, no cytopathic effect was observed in lentivector transduced cells infected with up to 10 ng of HIV. In contrast, the non-transduced cultures developed cytopathic effect with all tested amounts of HIV.

The applicability of the inhibitory effect on HIV growth to primary cells and its specificity for lentiviral vectors were tested in another experiment (see Figure 2). $CD4^+$ PBL's were transduced with either lentivector (pHR2-PGK-GFP) or the MLV retrovector carrying the same GFP transgene driven by the human PGK promoter, and sorted for transgene expression. The selected populations then were challenged with HIV virus as described above. Both the non-transduced cells (indicated in the figure by diamonds) and sorted cells transduced by the MLV retrovector (indicated by squares) yielded similar levels of p24 antigen in the culture medium. However, the cells transduced by the lentiviral (indicated by triangles) yielded sharply reduced p24 even after inoculation with high doses of HIV (100 ng p24 equivalent of virus). Moreover, there were twice as many cells transduced by the

lentivector surviving 13 days after infection than those transduced by the retrovector or non-transduced. In cells transduced by the lentivector, transgene expression was augmented significantly after infection with the HIV virus.

All publications and patent applications cited in this specification are herein incorporated by reference in their entirety as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

As will be apparent to those skilled in the art to which the invention pertains, the present invention may be embodied in forms other than those specifically disclosed above, for example to transfect and transduce other mammalian cell types, without departing from the spirit or essential characteristics of the invention. The particular embodiments of the invention described above, are, therefore, to be considered as illustrative and not restrictive. The scope of the present invention is as set forth in the appended claims rather than being limited to the examples contained in the foregoing description.

We claim:

1. A method for treating a host infected with a lentivirus comprising exposing said host to a lentivirus vector and a biologically acceptable carrier, excipient and diluent.

2. The method of claim 1, wherein said vector has an intact 5' LTR.

3. The method of claim 1, wherein said lentivirus is human immunodeficiency virus (HIV).

4. The method of claim 3, wherein said HIV is HIV-1.

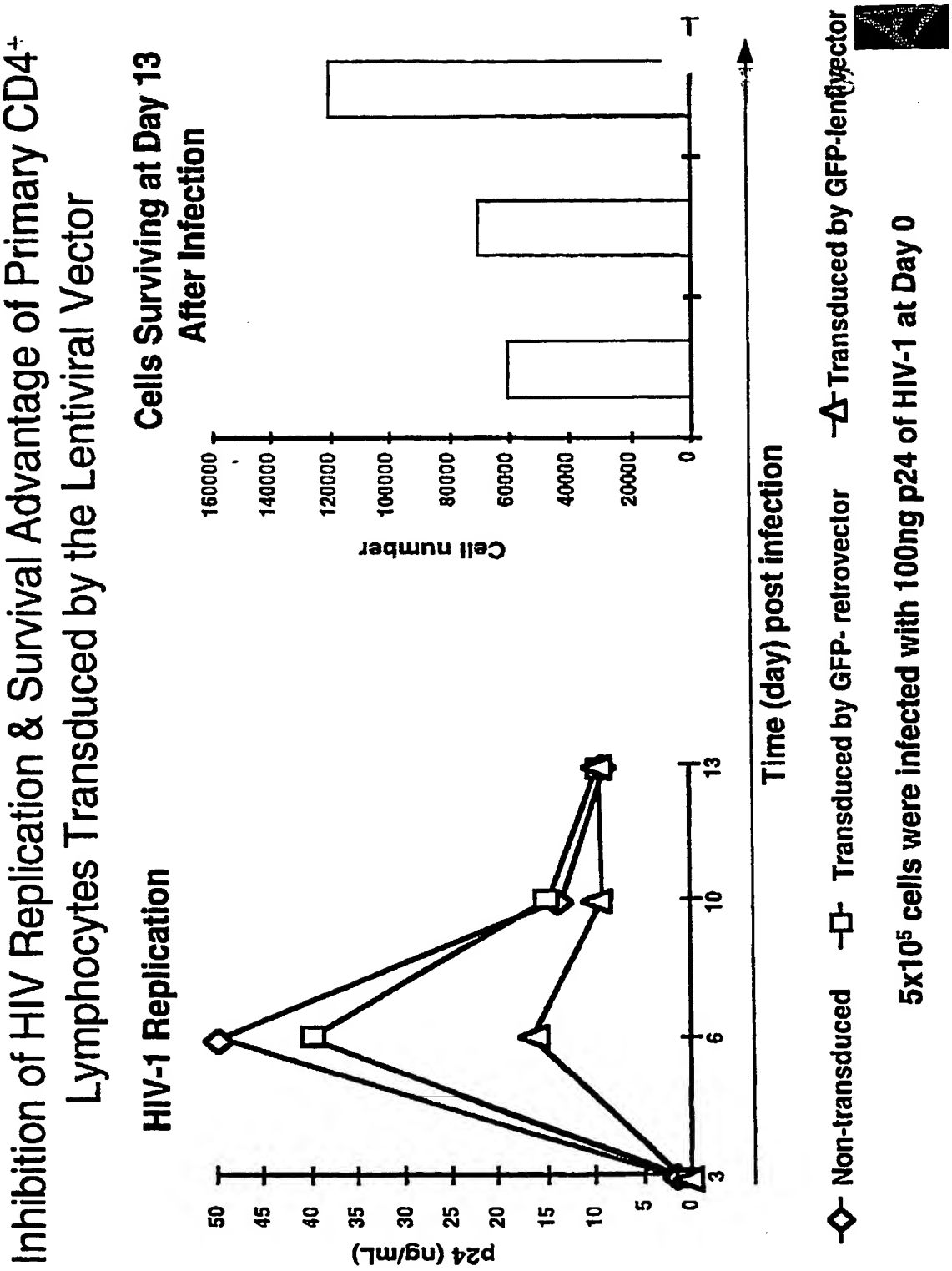

Inhibition of HIV Replication & Survival Advantage of Primary CD4+ Lymphocytes Transduced by the Lentiviral Vector
HIV-1 Replication
p24 (ng/mL)
50
45
40
35
30
25
20
15
10
5
0
3
6
10
13
Time (day) post infection
Cells Surviving at Day 13 After Infection
Cell number
160000
140000
120000
100000
80000
60000
40000
20000
0
T
Non-transduced
Transduced by GFP- retrovector
Transduced by GFP-lentivector
5x10⁵ cells were infected with 100ng p24 of HIV-1 at Day 0

Figure 2

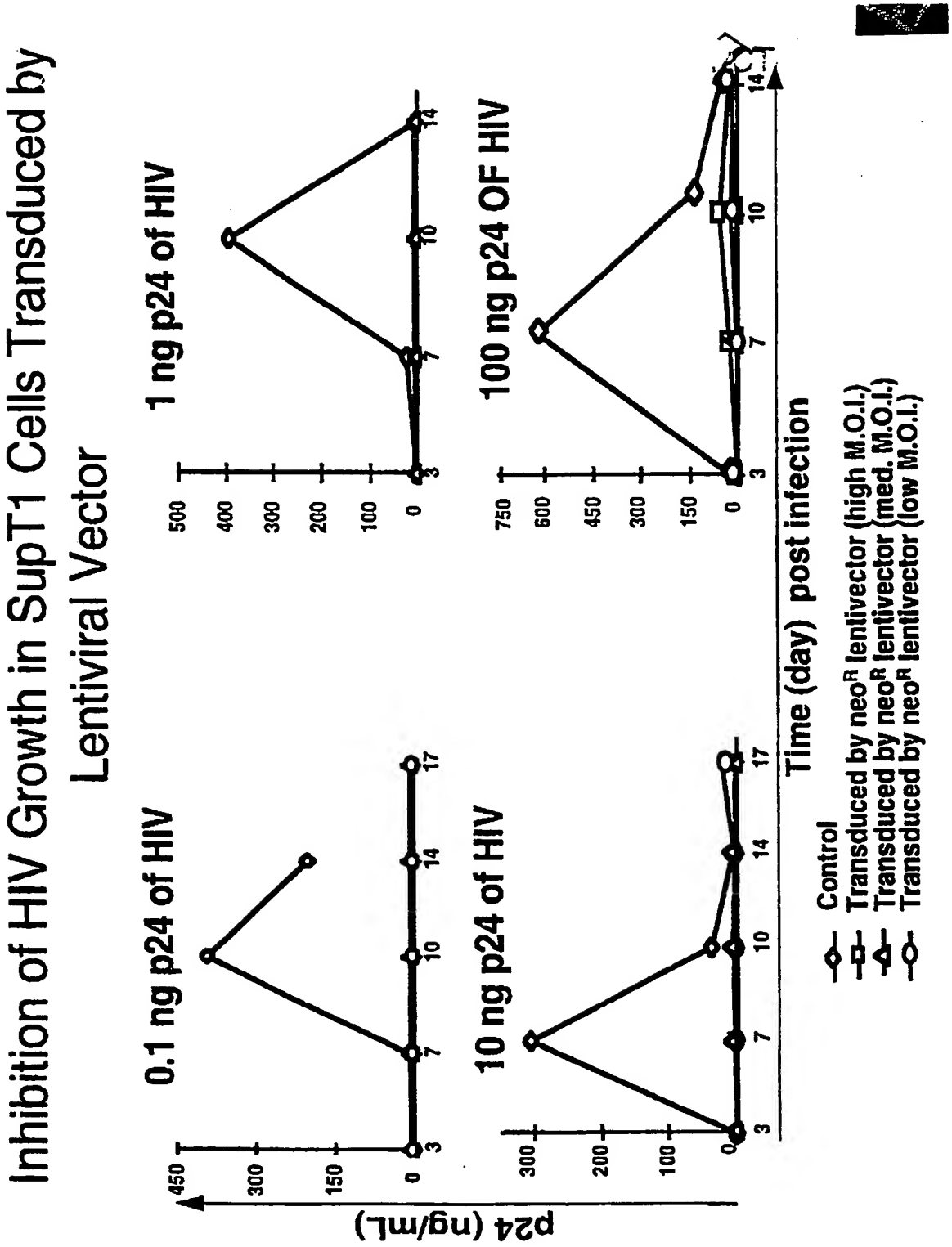

Inhibition of HIV Growth in SupT1 Cells Transduced by Lentiviral Vector
0.1 ng p24 of HIV
1 ng p24 of HIV
10 ng p24 of HIV
100 ng p24 OF HIV
p24 (ng/mL)
Time (day) post infection
Control
Transduced by neoR lenlivector (high M.O.I.)
Transduced by neoR lenlivector (med. M.O.I.)
Transduced by neoR lenlivector (low M.O.I.)

Figure 1

SEQUENCE LISTING

<110> Song, Jin-Ping
Naldini, Luigi
Cell Genesys

<120> THERAPEUTIC USE OF LENTIVIRAL VECTORS

<130> F126422

<140>
<141>

<150> 60/069,579
<151> 1997-12-12

<160> 2

<170> PatentIn Ver. 2.0

<210> 1
<211> 65
<212> DNA
<213> primer

<400> 1
ccatcgatca cgagactagt cctacgtatc cccggggacg ggatccgcgg aattccgttt 60
aagac 65

<210> 2
<211> 20
<212> DNA
<213> primer

<400> 2
ttataatgtc aaggcctctc 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25720

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 48/00, 35/00; C12N 15/63
US CL :514/44; 424/93.1; 435/320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/93.1; 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,650,309 A (WONG-STAAL et al.) 22 July 1997, see entire document, especially column 2 and column 15, lines 54-60.	1-4
X	LISZIEWICZ et al. Inhibition of human immunodeficiency virus type 1 replication by regulated expression of a polymeric Tat activation response RNA decoy as a strategy for gene therapy in AIDS. PNAS USA, September 1993, Vol. 90, pages 8000-8004, see entire document.	1-4
A	BERKHOUT et al. Tat trans-activates the human immunodeficiency virus through a nascent RNA target. Cell 20 October 1989, Vol. 59, pages 273-282, entire document.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 FEBRUARY 1999	Date of mailing of the international search report 25 FEB 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ANNE-MARIE BAKER, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25720

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS
DIALOG (file:medicine, USPatFull, Derwent, European Patents, JAPIO)
search terms: HIV, lentivir?, retrovir?, vector?, LTR, treat?, infect?

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: F126422

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US98/25720	11 December 1998 (11.12.98)	12 December 1997 (12/12/97)

Title of invention:
THERAPEUTIC USE OF LENTIVIRAL VECTORS

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

NALDINI, Luigi
Corso Monte Cucco 144
10141 Torino, Italy

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IT	IT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SONG, Jin-Ping
3455 Rambow Dr.
Palo Alto, California 84306, US

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CN	US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/US98/25720

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [XX] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

NAKAMURA, Dean H.
MACK, Susan J.
SEAS, Robert J.
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 Pennsylvania Ave., N.W., Suite 800
Washington, D.C. 20037-3213, US

Telephone No.: 202/293-7060

Facsimile No.: 202/293-7860

Teleprinter No.:

[ ] Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [X] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[X] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Sheet No. 3

International application No.
PCT/US98/25720

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:	2	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter	:		sheets	☐	☐
6. other *(specify)* formal drawings Substitute Sheets 3 and 3-1	:	2 2	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [X] other *(specify)*: Check

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Dean H. Nakamura, Reg. No. 33,981

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No. PCT/US98/25720	For International Preliminary Examining Authority use only
Applicant's or agent's file reference F126422	Date stamp of the IPEA

Applicant

NALDINI, Luigi et al

Calculation of prescribed fees

1. Preliminary examination fee	490.00	P
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	162.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	652.00	TOTAL

Mode of Payment

[xx]	authorization to charge deposit account with the IPEA (see below)	[ ]	cash
[xx]	cheque	[ ]	revenue stamps
[ ]	postal money order	[ ]	coupons
[ ]	bank draft	[ ]	other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ US [ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

19-4880	24/06/99	
Deposit Account Number	Date *(day/month/year)*	Signature

See Notes to the fee calculation sheet

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: DEAN H. NAKAMURA
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 PENNSYLVANIA AVENUE, N.W., SUITE 800
WASHINGTON DC 20037-3213

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	26 JUL 2000
Applicant's or agent's file reference F126422	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US98/25720	11 DECEMBER 1998	12 DECEMBER 1997

Applicant
NALDINI, LUIGI

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Anne-Marie Baker, Ph.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference F126422	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/25720	International filing date *(day/month/year)* 11 DECEMBER 1998	Priority date *(day/month/year)* 12 DECEMBER 1997
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 48/00, 35/00; C12N 15/63 and US Cl.: 514/44; 424/93.1; 435/320.1		
Applicant NALDINI, LUIGI		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24 JUNE 1999	15 JUNE 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Anne-Marie Baker, Ph.D. Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25720

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25720

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	NONE	YES
	Claims	1-4	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-4	NO
Industrial Applicability (IA)	Claims	1-4	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by U.S. Patent No. 5,650,309.

The claims are directed to a method for treating a host infected with a lentivirus by exposing the host to a lentivirus vector. The claims are more particularly drawn to the use of a vector having an intact 5' LTR, especially HIV-1.

U.S. Patent No. 5,650,309 discloses vectors which stably transduce cells, rendering the cells resistant to a target virus. The vectors are amplified upon infection of the cell by a target virus, and spread throughout an infected host in response to infection by the target virus. The invention provides vectors constructed to afford two levels of anti-viral activity once transduced into a biological host cell. The first level of anti-viral activity is provided by an anti-viral agent encoded by the vector which is transduced into the host cell. The secondary protective effect is produced upon infection of the host cell by the target virus, which causes the nucleic acid encoding the anti-viral agent to be replicated and encapsidated into viral particles, which then deliver the nucleic acid encoding the anti-viral agent to other cells within the host, thereby blocking or ameliorating infection by the target virus (Column 2). The invention incorporates the use of the HIV LTR promoter, wherein the anti-HIV agent is operably linked to the HIV LTR promoter, and expressed upon infection by active HIV, thereby suppressing infection by replication competent HIV viruses (Column 13, lines 56-59). When the anti-HIV gene is under the control of the HIV 5' LTR, the activating gene (tat) can be introduced by infection with HIV, or by transfection with another vector carrying the gene (Column 15, lines 54-60). Thus, U.S. Patent No. 5,650,309 discloses all the instantly claimed embodiments.

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by Lisziewicz et al. (1993).

The claims are directed to a method for treating a host infected with a lentivirus by exposing the host to a lentivirus vector, particularly a vector having an intact 5' LTR, especially HIV-1.

Lisziewicz et al. (1993) disclose a replication-defective (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-19, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the claims,
page(s) 20, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

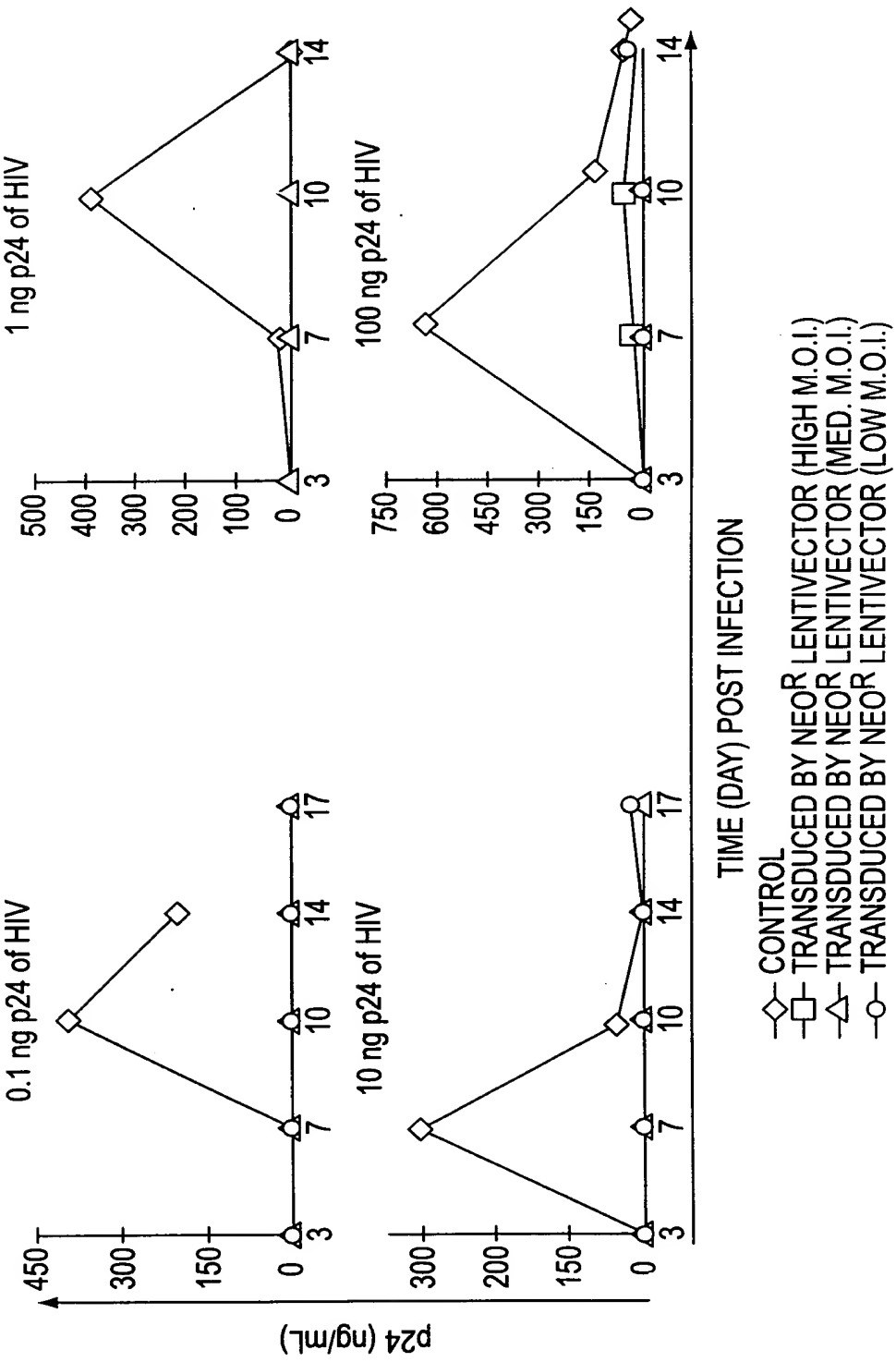
This report has been drawn on the basis of the drawings,
page(s) NONE, as originally filed.
page(s) 1-2, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) 1, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
retroviral vector comprising an HIV-LTR driven 50TAR construct. The report discloses a method for treating HIV-1 infection by intracellular expression of an RNA decoy and ribozyme. The RNA decoy, consisting of polymeric Tat activation response elements (TARs), is designed to compete for Tat binding in an equilibrium with viral TAR RNA, thereby inhibiting viral replication. The expression of polymeric TAR is regulated by the HIV LTR and transcriptional activation is dependent on the presence of HIV Tat. Plasmids expressing up to 50 tandem copies of TAR RNA (50TAR) inhibited tat-mediated gene expression by more than 90% in a transient transfection assay. In addition, a gag RNA-specific ribozyme gene was introduced into the 50TAR containing retroviral vector to enhance the inhibitory effect of the construct (TAR-Rib). A human T cell line was infected with the TAR-Rib recombinant retrovirus and challenged with HIV-1. HIV-1 replication was inhibited by 99% in the TAR-Rib-transduced T cells and was maintained over a 14-month period. Thus, Liszlewicz et al. disclose all the instantly claimed embodiments.

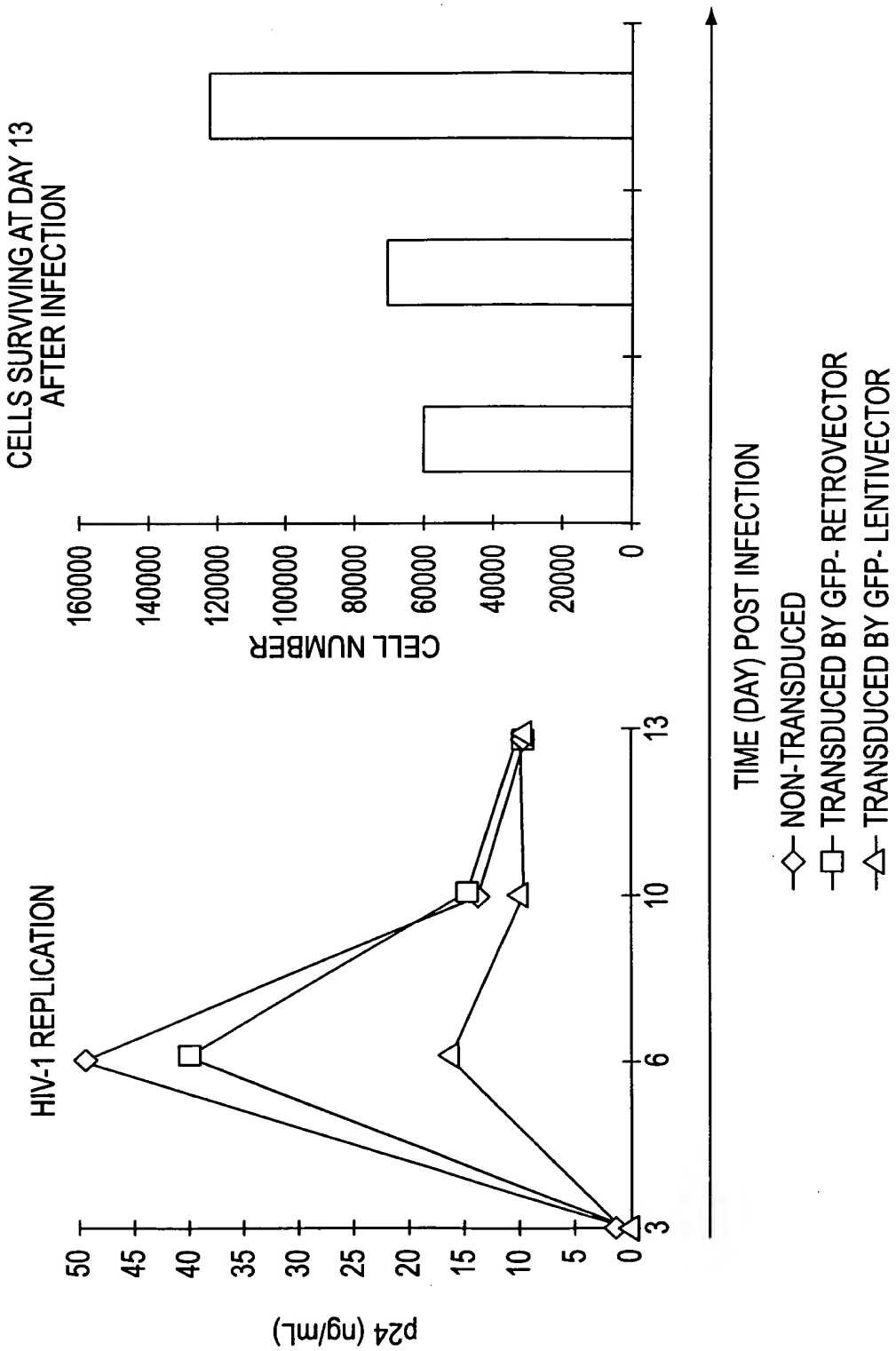
---------- NEW CITATIONS ----------
NONE

FIG. 1



PCT/US98/25720
IPEA/US 24 JUN 1999
ART 34 AMDT

FIG. 2



PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: DEAN H. NAKAMURA
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 PENNSYLVANIA AVENUE, N.W., SUITE 800
WASHINGTON DC 20037-3213

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	09 MAR 2000
Applicant's or agent's file reference: F126422	**REPLY DUE** within ONE months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/25720	11 DECEMBER 1998	12 DECEMBER 1997

International Patent Classification (IPC) or both national classification and IPC
IPC(7): A61K 48/00, 35/00; C12N 15/63 and US Cl.: 514/44; 424/93.1; 435/320.1

Applicant
NALDINI, LUIGI

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 12 APRIL 2000

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	ANNE-MARIE BAKER, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (January 1994)★

WRITTEN OPINION

International application No.
PCT/US98/25720

I. Basis of the opinion

1. This opinion has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

☐ the international application as originally filed.

☒ the description, pages 1-19 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.

☒ the claims, Nos. 1-4 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.

☒ the drawings, sheets/~~fig~~ NONE , as originally filed.
sheets/~~fig~~ 1-2 , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:
NONE

WRITTEN OPINION

International application No.
PCT/US98/25720

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	NONE	YES
	Claims	1-4	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-4	NO
Industrial Applicability (IA)	Claims	1-4	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by U.S. Patent No. 5,650,309.

The claims are directed to a method for treating a host infected with a lentivirus by exposing the host to a lentivirus vector. The claims are more particularly drawn to the use of a vector having an intact 5' LTR, especially HIV-1.

U.S. Patent No. 5,650,309 discloses vectors which stably transduce cells, rendering the cells resistant to a target virus. The vectors are amplified upon infection of the cell by a target virus, and spread throughout an infected host in response to infection by the target virus. The invention provides vectors constructed to afford two levels of anti-viral activity once transduced into a biological host cell. The first level of anti-viral activity is provided by an anti-viral agent encoded by the vector which is transduced into the host cell. The secondary protective effect is produced upon infection of the host cell by the target virus, which causes the nucleic acid encoding the anti-viral agent to be replicated and encapsidated into viral particles, which then deliver the nucleic acid encoding the anti-viral agent to other cells within the host, thereby blocking or ameliorating infection by the target virus (Column 2). The invention incorporates the use of the HIV LTR promoter, wherein the anti-HIV agent is operably linked to the HIV LTR promoter, and expressed upon infection by active HIV, thereby suppressing infection by replication competent HIV viruses (Column 13, lines 56-59). When the anti-HIV gene is under the control of the HIV 5' LTR, the activating gene (tat) can be introduced by infection with HIV, or by transfection with another vector carrying the gene (Column 15, lines 54-60). Thus, U.S. Patent No. 5,650,309 discloses all the instantly claimed embodiments.

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by Lisziewicz et al. (1993).

The claims are directed to a method for treating a host infected with a lentivirus by exposing the host to a lentivirus vector, particularly a vector having an intact 5' LTR, especially HIV-1.

Lisziewicz et al. (1993) disclose a replication-defective (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
retroviral vector comprising an HIV-LTR driven 50TAR construct. The report discloses a method for treating HIV-1 infection by intracellular expression of an RNA decoy and ribozyme. The RNA decoy, consisting of polymeric Tat activation response elements (TARs), is designed to compete for Tat binding in an equilibrium with viral TAR RNA, thereby inhibiting viral replication. The expression of polymeric TAR is regulated by the HIV LTR and transcriptional activation is dependent on the presence of HIV Tat. Plasmids expressing up to 50 tandem copies of TAR RNA (50TAR) inhibited tat-mediated gene expression by more than 90% in a transient transfection assay. In addition, a gag RNA-specific ribozyme gene was introduced into the 50TAR containing retroviral vector to enhance the inhibitory effect of the construct (TAR-Rib). A human T cell line was infected with the TAR-Rib recombinant retrovirus and challenged with HIV-1. HIV-1 replication was inhibited by 99% in the TAR-Rib-transduced T cells and was maintained over a 14-month period. Thus, Lisziewicz et al. disclose all the instantly claimed embodiments.

---------- NEW CITATIONS ----------
NONE

Inhibition of HIV Replication & Survival Advantage of Primary CD4+ Lymphocytes Transduced by the Lentiviral Vector

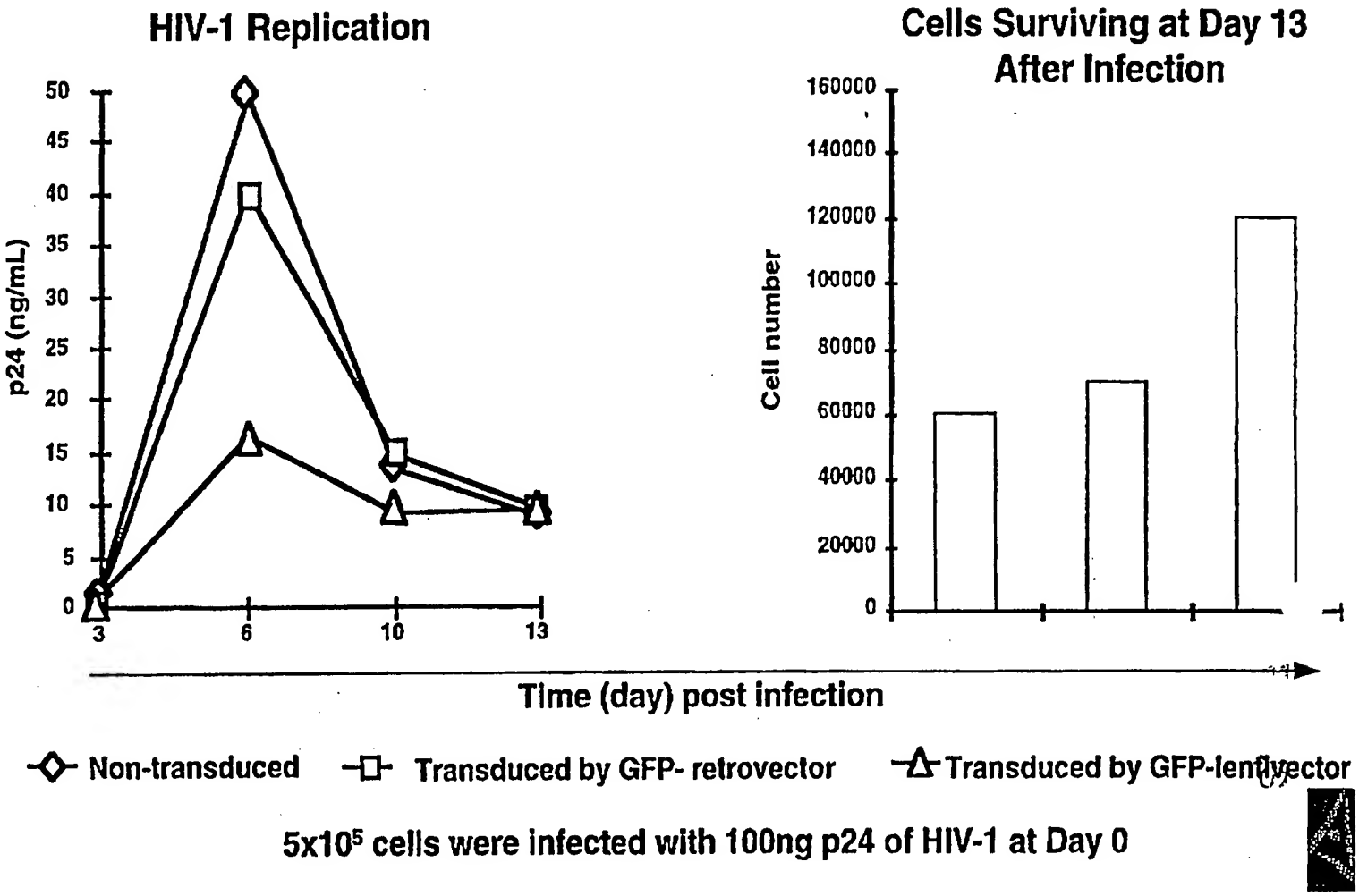


Figure 2

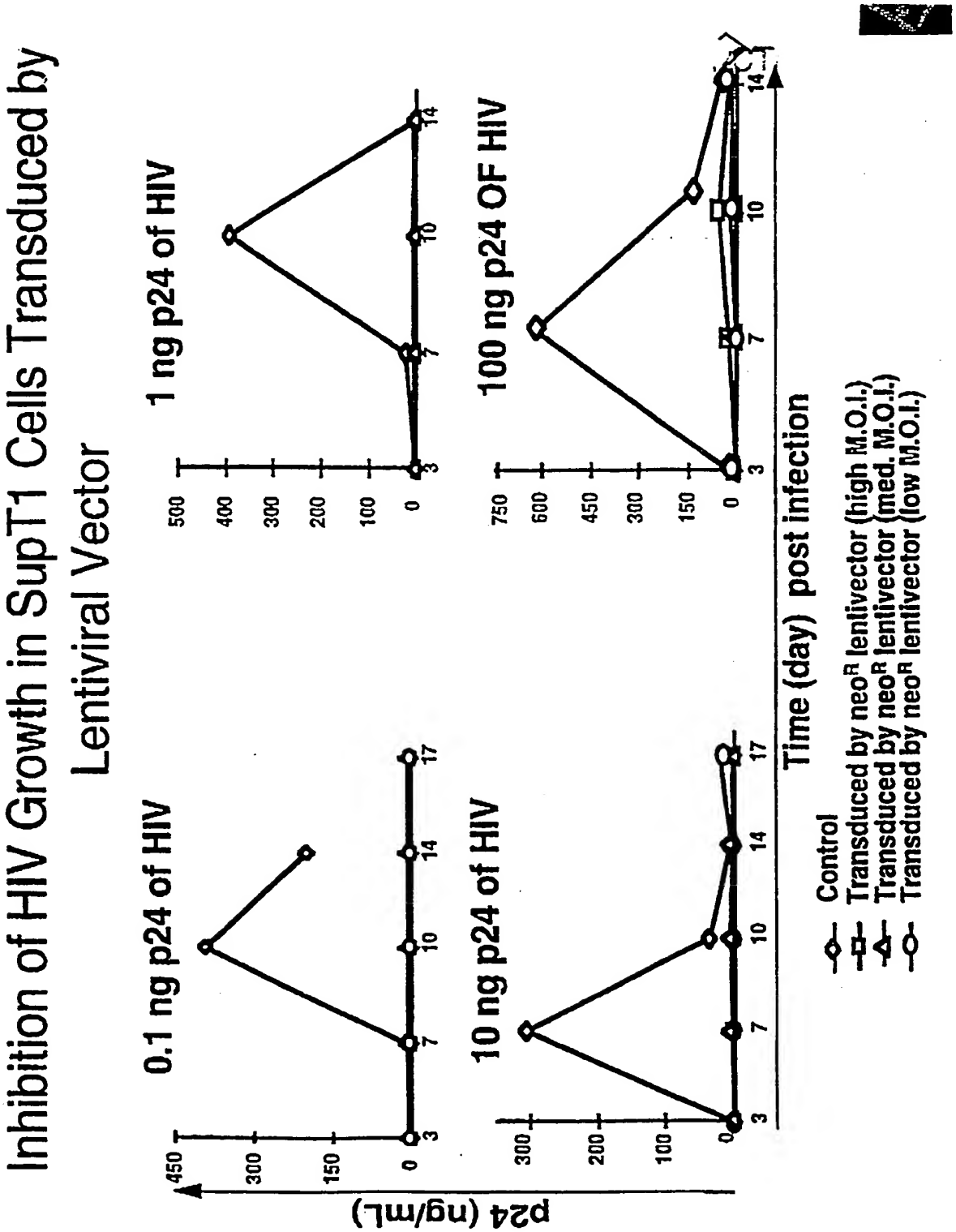

Inhibition of HIV Growth in SupT1 Cells Transduced by Lentiviral Vector
0.1 ng p24 of HIV
1 ng p24 of HIV
10 ng p24 of HIV
100 ng p24 OF HIV
p24 (ng/mL)
Time (day) post infection
Control
Transduced by neoR lentivector (high M.O.I.)
Transduced by neoR lentivector (med. M.O.I.)
Transduced by neoR lentivector (low M.O.I.)

Figure 1